HMW DNA extraction from diverse plants species for PacBio and Nanopore sequencing

Russo, Alessia ; Potente, Giacomo ; Mayjonade, Baptiste

Abstract: Modified from the protocol of Baptiste Mayjonade, Jérome Gouzy, Cécile Donnadieu, Nicolas Pouilly, William Marande, Caroline Callot, Nicolas Langlade and Stéphane Munos, High molecular weight gDNA extraction, Bio Techniques, Vol. 61, No. 4, October 2016, pp. 203-205. BioTechniques 61:203-205 (October 2016) doi 10.2144/000114460 https://www.future-science.com/doi/10.2144/000114460 The original protocol failed to extract High Molecular Weight genomic DNA from orchids samples (poor yield and low purity). Therefore modifications (mainly/mostly BME addition during the lysis step and a Phenol:Chloroform purification step) were made to create a new version of this protocol. This new version allowed to successfully extract DNA from orchid samples. This DNA was then sequenced on a Nanopore PromethION platform and good results in term of total yield and read length were obtained. This protocol was also successfully applied to a wide range of plant species (See attached document/excel sheet).

DOI: https://doi.org/10.17504/protocols.io.5t7g6rn

Apr 12, 2021

HMW DNA extraction from diverse plants species for PacBio and Nanopore sequencing

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1 Works for me dx.doi.org/10.17504/protocols.io.5t7g6rn

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ABSTRACT

Modified from the protocol of Baptiste Mayjonade, Jérome Gouzy, Cécile Donnadieu, Nicolas Pouilly, William Marande, Caroline Callot, Nicolas Langlade and Stéphane Munos, High molecular weight gDNA extraction, Bio Techniques, Vol. 61, No. 4, October 2016, pp. 203-205. BioTechniques 61:203-205 (October 2016) doi 10.2144/000114460
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The original protocol failed to extract High Molecular Weight genomic DNA from orchids samples (poor yield and low purity). Therefore modifications (mainly/mostly BME addition during the lysis step and a Phenol:Chloroform purification step) were made to create a new version of this protocol. This new version allowed to successfully extract DNA from orchid samples. This DNA was then sequenced on a Nanopore PromethION platform and good results in term of total yield and read length were obtained. This protocol was also successfully applied to a wide range of plant species (See attached document/excel sheet).

ACKNOWLEDGEMENT

Sequencing of the *Arabidopsis thaliana* samples listed in the attached reports was performed in collaboration with the GeT core facility, Toulouse, France (http://get.genotoul.fr), and was supported by France Génomique National infrastructure, funded as part of “Investissement d’avenir” program managed by Agence Nationale pour la Recherche (contract ANR-10-INBS-09). We particularly want to thank Lisa Gil and Céline Lopez-Roques for the sequencing part and Roxane Boyer for the data processing.

Sampling and DNA Extraction for the *Ophrys sphegodes* samples were financially supported by the University of Zürich Research Priority Programme URPP "Evolution in Action" (PMS/AR). Sequencing was performed by Daniel Frei in the Department of Molecular Diagnostics, Genomics and Bioinformatics, Agroscope Wädenswil, Switzerland. We are thankful to Jürg E. Frey, Agroscope, for financial support. We are grateful to Philipp M. Schlüter and Ueli Grossniklaus for support and helpful discussion.

Sequencing of *Lolium multiflorium* was performed in Agroscope by Daniel Frei. We thank Daniel, Jürg E. Frei, Dario Copetti and Bruno Studer, Department of Environmental Systems Science, ETH Zürich, for sharing the sequencing results.

Sequencing of *Brassica incana* was performed at FGCZ Zürich. We are thankful to Léa Frachon, Department of Systematic and Evolutionary Botany, UZH Zürich, to share the data.

DNA extraction of *Gorteria diffusa* was performed by Roman T. Kellenberger, Department of Plant Sciences, University of Cambridge. We wish to thank him for sharing his sequencing results.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Russo A., Potente P., Mayjonade B., HMW DNA Extraction from diverse plant species for PacBio and Nanopore sequencing, protocols.io, 2021. doi: dx.doi.org/10.17504/protocols.io.5t7g6rn

ATTACHMENTS

Sample sequencing reports & validated species.xlsx
report_ONT_PromethION_arabidopsis_1.pdf
report_ONT_PromethION_arabidopsis_2.pdf
report_ONT_PromethION_Ophrys_1.pdf
report_ONT_PromethION_Ophrys_2.pdf

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KEYWORDS

plant DNA extraction, DNA sequencing, long reads, PromethION, plant genomics, PacBio, HMW DNA, Nanopore

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GUIDELINES

Presence of ß-mercapto-ethanol, Phenol and Chloroform. Work under a fume hood!

MATERIALS TEXT

MATERIALS

Buffer

EB **Qiagen Catalog #19086**

SeraMag SpeedBeads Carboxyl-Magnet-Beads hydrophob **Fisher Scientific Catalog #GE Healthcare 65152105050250**

RNAse A (DNAse free) 100mg/ml

Qiagen Catalog #19101

REAGENTS

- Sodium Chloride Biotechnology Grade
- TRIS pH 8.0 1 M Biotechnology Grade
- EDTA 0.5 M sterile solution
- SDS 20% Biotechnology Grade
- PVP WT 40000
- Sodium Metabisulfite
- Potassium Acetate
- PEG 8000
- RNase DNase-free! 100mg/ml
- Absolute Ethanol
- β-mercapto-ethanol
- Ready-to-use Phenol:Chloroform:Isoamylalcohol pH=8 (25:24:1, v/v)
  OR
- Phenol pH=8
- Chloroform:Isoamylalcohol (24:1, v/v)

EQUIPMENT

Magnetic Stand
Magnetic Stand

Thermo Scientific **MR02**

Any magnetic rack that fits your tubes will suffice.

Mini Tube Rotator

FisherBrand **15534080**

0.10ml mini sampling spoon

Bel-Art SP Scienceware **-**

REF: 367210010

- Thermomixer/Water Bath
- Mortar & pestle
- Centrifuge for 2ml tubes

SAFETY WARNINGS

Work under fume hood when add β-mercapto-ethanol, Phenol and Chloroform.

BEFORE STARTING

- Prepare **the Bead Solution (you can prepare it and keep it at 4 °C for weeks)**

Follow this protocol for beads preparations: https://www.protocols.io/view/dna-size-selection-3-4kb-and-purification-of-dna-u-n7hdhj6/abstract

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Ramawatar, Benjamin Schwessinger. DNA size selection (>3-4kb) and purification of DNA using an improved homemade SPRI beads solution..
http://dx.doi.org/10.17504/protocols.io.n7hdhj6

N.B.: In this protocol, we omit the use of Tween-20.

SeraMag SpeedBeads Carboxyl-Magnet-Beads hydrophob **Fisher Scientific Catalog #GE Healthcare 65152105050250**

A	B	C	D
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 10 ml**
Tris HCL pH=8	1 M	10 mM	100 µl
EDTA pH=8	0.5 M	1 mM	20 µl
NaCl	5 M	1.6 M	3.2 ml
PEG 8000	50% (w/v)	11%	2.2 ml
Washed Sera-Mag beads	100%	0.40%	40 µl
Milli-Q Water			up to 10 ml

- WARNING --> If PEG 8000 is too old, it could compromise binding of beads to DNA.

1. Combine Water, Tris-HCl, EDTA, and NaCl in a 50 ml tube.
2. Vortex the SeraMag Beads thoroughly (ensuring that all the beads are resuspended and nothing is left at the bottom of the tube) and pipette 40 µl into a 1.5 ml tube, place it on the magnetic rack and wait until the solution has cleared up and all the beads have bound at the back of the tube.
3. Remove supernatant and add 1ml Milli-Q Water.
4. Take tube out of the magnet, vortex it, spin it down and put it back on the magnet.
5. Wait until all beads bind at the back of the tube.
6. Repeat the washing step (3 - 5) 3 more times.
7. Remove supernatant.
8. Take the tube off the magnet and add 40 µl of the previously prepared (step 1) stock solution, vortex it and spin down. Pipette everything back to remaining stock solution previously prepared (step 1).
9. Slowly pipette 2.2 mL of 50% PEG into the 50 mL tube. Vortex.
10. Add Milli-Q Water up to 10 ml.

- **Potassium Acetate 5M**

Dissolve 4.9 gr KAc in 10 ml ddH_2O (4.9 gr KAc + ≈ 7.5 ml H_2O).

- Pre-cool mortar and pestle in the freezer at -80 °C

DAY OF THE EXPERIMENT

- Incubate Lysis Buffer at 65 °C for at least 30 minutes (gently mix the tube every 10 minutes). After that, you can already prepare aliquotes of 600 µl (one per sample).
- Place Phenol at RT to equilibrate (at least 1 h).
- Prepare incubator/water bath at 55 °C.
- Incubate the bead solution at RT for 30 minutes prior to use.
- Prepare liquid N_2 and plant tissue.

- Prepare the **SDS Lysis Buffer**

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A	B	C	D
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 10 ml**
PVP40	100%	1%	0.1 gr
Sodium Metabisulfite	1%	1%	0.1 gr
NaCl	2.5 M	0.5 M	2 ml
Tris HCl pH 8	1 M	100 mM	1 ml
EDTA pH 8	0.5 M	50 mM	1 ml
β-mercapto-ethanol		2%	200 µl
H2O Mol Biol Grade			up to 10 ml (≈ 5.05 ml)
SDS	20%	1.5%	750 µl

When preparing the SDS Lysis Buffer, add SDS at last, after having added all the other reagents and having mixed the solution by vortexing. This will help avoiding bubble formation.

β-mercapto-ethanol (BME) is optional, and for some plant species sodium metabisulfite is sufficient to prevent polyphenols oxidization. However, BME is a strong antioxidant and a reducing agent which could also play a role in the protection of DNA.

- **Washing solution (EtOH 70%), fresh**

35 ml EtOH 100% + 15 ml H_2O

Cell Lysis 1h

1 Incubate Lysis Buffer at 65 °C for at least 30 minutes (gently mix the tube every 10 minutes). For convenience, one can aliquote 600 µl Lysis Buffer in a 2 ml tube per sample and incubate in the water bath. 30m
Keep the SDS Lysis Buffer at 65°C until the tissue powder is added (the buffer has to be warm to ensure that powder is well dissolved).

Work under a fume hood until the end of the protocol because of the presence of BME, Phenol and Chloroform.

2 ⚠

Grind ~100-110 mg leaf tissue in LN_2 with mortar and pestle. This is a crucial step as it will affect final DNA yield, purity, and integrity.
Plant leaves must be flash frozen in LN_2 immediately after harvesting and stored at -80 °C until DNA extraction procedure, if not immediately used.
Here a useful tutorial on how to grind plant tissue with LN_2 https://bionanogenomics.com/videos/liquid-nitrogen-grinding-tutorial/ . As they say: "Keep grinding until powder is as fine as wheat flour"
Tips to achieve better results:
- Apply enough pressure to allow cells to break and recover as much DNA as possible.
- Keep the powder frozen until it goes into the lysis buffer, by adding LN2 while grinding (LN2 evaporates quickly). This prevents nuclease activation and oxidation processes (once powder is in the lysis buffer, the nucleases will be inactivated by the SDS and EDTA, and the oxidation blocked by BME and Metabisulfite)

3 Carefully add ~ 50-100 mg tissue powder (1-2 spoons SP SCIENCEWARE 0.10 ml, see equipment list) to a 2 ml tube with 600 µl of warm SDS Lysis Buffer.

Add more than 100 mg of tissue can decrease DNA purity (A260/230).

Immediately homogenize by vortexing 3-5 seconds. It's important to rapidly homogenize the lysate to neutralize DNase, polyphenols,...

4 Incubate 20 min @ 55°C with **gentle agitation** in a thermomixer (maximum 400 rpm) or in a water bath. Invert the tube gently 10 times after 10 min. 20m

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5 Add 4 µl 100mg/ml RNase A, gently mix by inversion 10 times and incubate 10 minutes at 55 °C with gentle agitation. 10m

Contaminant precipitation and phenol:chloroform purification 40m

6 Add 200 µl 5M KAc. Mix by gently pipetting up and down 5 times with a P1000 cut tip and by inverting the tube 5 times in order to obtain a homogeneous solution to fully precipitate the proteins and the polysaccharides that will complex with SDS.

7 Phenol:Chloroform purification.
Option 1: Add 800 µl Phenol:Chloroform:IsyamylAlcohol pH=8.
Option 2: If you don't have a ready-to-use Phenol:Chloroform:IsyamylAlcohol solution, add 400 µl Phenol pH=8, mix by inverting and add 400 µl Chloroform:IsoamylAlcohol 24:1.

Work under a fume hood.

8 Incubate 10 min @ 20rpm/min on a rotator. 10m

9 Centrifuge 10 minutes @ 10,000g at RT. 10m

10 Transfer the supernatant (usually 700-800 µl) in a new 2 ml tube with a P1000 cut tip. Pipette slowly and with a cut tip to avoid breaking the long DNA fragments. Make sure not to carry over the interphase or the lower layer that would contaminate the DNA.

11 Add the same volume of Chloroform:IsoamylAlcohol 24:1

12 Incubate at RT for 10 min with gentle agitation (20 rpm) 10m

13 Centrifuge RT 10,000g for 10 min 10m

14 Transfer the supernatant into a new 2 ml tube. Make sure not to carry over the interphase or lower layer that would contaminate the DNA.

Determine the final volume.

Genomic DNA Purification 1h 30m

15 Before starting, vortex the Bead Solution for 30 seconds to ensure beads are completely resuspended.

Add 1 volume of Bead Solution to the sample tube (1:1 ratio, v/v).

16 Incubate @10 rpm on a rotator for 20-30 min at RT. 30m

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17 Spin down for 1 second.

18 Place the tube on a magnetic rack and wait until all beads bind at the back of the tube and the solution becomes clear (this will probably take a few minutes, as the DNA is highly concentrated and HMW). 10m

19 Remove the supernatant without disturbing the bead pellet.

20 Add 1 ml EtOH 70% (the washing solution).

21 Remove the tube from the magnetic rack and mix by gently inverting the tube 20 times to resuspend the bead pellet in the washing solution (probably it won't be entirely resuspendend and there will be some small/big bead aggregates floating - if that happens, don't worry).

22 Spin down for 1 second.

23 Place the tube on a magnetic rack and wait until all beads bind at the back of the tube and the solution becomes clear.

24 Remove the supernatant without disturbing the bead pellet.

25 Repeat steps 20 - 24 two-three times (total possible washing = 4 times, but usually 2 washes are enough).

26 Spin down for 1 second and remove the remaining washing solution with a P10 pipette.

27 Add 50 µl EB (or Tris-HCl 10 mM pH=8.5).

28 Resuspend the beads by gently swirling or flicking the tube. This time they must be completely resuspended. It is important that the beads are not aggregated, or the elution efficiency will be reduced.

29 Incubate at 37 °C for 15 minutes and flick the tube again. 15m

30 Spin down for 1 second.

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31 Place the tube on a magnetic rack and wait until all beads bind at the back of the tube and the solution becomes clear. 30m
If DNA is at high concentration, this can take a long time. You can leave it overnight at RT, and/or add more EB (if you do so mix by flicking the tube after the addition of EB and put the tube back on the Magnetic Rack).

32 Transfer the eluted DNA into a new tube, with a cut tip, or using a wide-bore tip, as DNA is very concentrated and the solution very viscous.

33 Leave the DNA at RT overnight, as the HMW DNA is very aggregated and needs to get relaxed and homogenized. The day after proceed with Nanodrop lecture, to determine the purity, and Qubit to determine concentration.

Additional Purification

34 An additional purification steps can improve the A260/A280 and A260/A230 ratios. At this step you can pool the elution of multiple tubes.
Before starting:
- Incubate the Beads Solution at RT for more than 30 minutes prior to use;
- Mix the beads by vortexing for 20 seconds before use .

Repeat steps 15 to 33.

QC

35 Determine the purity of the DNA at 230, 260, 280 nm with a spectrophotometer (e.g. NanoDrop 2000). Acceptable values are:
- A260/280 = 1.8 - 2.0;
- A260/230 = 2.0 - 2.2.

A260/280 >2.0 or A260/230 >2.2 could be indicative of a contamination by RNA.

36 Determine the concentration of double-stranded DNA with a fluorimetric-based method (e.g. Qubit 3.0 Fluorometer BR assay). The ratio between the concentration measured with Nanodrop, over the concentration measured by Qubit should be <2.

37 Evaluate DNA integrity on a TapeStation, and fragment length on PhemtoPulse (or PFGE).

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